GC634-2
9/17/01
Bron et al

FIGURES

Fig. 1.

A

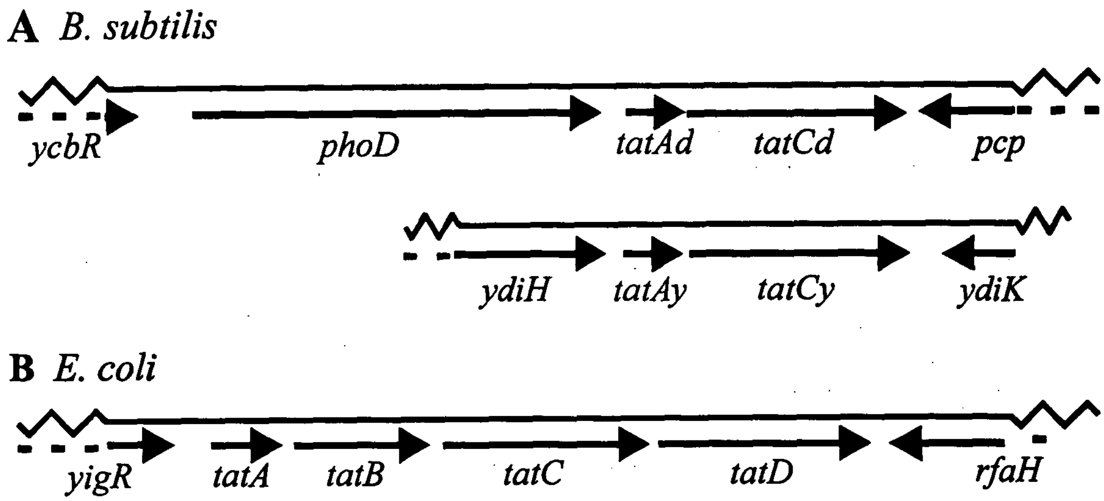
```
TatA(Eco)   M-GGISIWQLLIIAVIVVLLFGTKKLG----------------------------   26
TatE(Eco)   M-GEISITKLLVVAALVVLLFGTKKLR----------------------------   26
TatAy(Bsu)  M--PIGPGSLAVIAIVALIIFGPKKLP----------------------------   25
TatAd(Bsu)  MFSNIGIPGLILIFVIALIIFGPSKLP----------------------------   27
TatAc(Bsu)  M--ELSFTKILVILFVGFLVFGPDKLP----------------------------   25
TatB(Eco)   MF-DIGFSELLLVFIIGLVVLGPQRLPVAVKTVAGWIRALRSLATTVQNELTQELKLQ   49
            *    ..   . ..   .   ...*.  .*

TatA(Eco)   ---------------SIGSDLGASIKGFKKAMSDDE----PKQDKTSQDADFTAKTI   64
TatE(Eco)   ---------------TLGGDLGAAIKGFKKAMNDDD----A-AAKKGADVDLQAEKL   63
TatAy(Bsu)  ---------------ELGKAAGDTLREFKNATKGLT----SDEEEKKKEDQ------   57
TatAd(Bsu)  ---------------EIGRAAKRTLLEFKSATKSLV----SGDEKEEKSAELTAVK-   64
TatAc(Bsu)  ---------------ALGRAAGKALSEFKQATSGLT----QDIRKNDSEN-----K-   57
TatB(Eco)   EFQDSLKKVEKASLTNLTPELKASMDELRQAAESMKRSYVANDPEKASDEAHTIHNP  114
                           ... .   .. ... * ..          .. .

TatA(Eco)   ADKQADTNQE-----------------------------QAKTEDAKRHDKEQV   89
TatE(Eco)   SHKE--------------------------------------------------   67
TatAy(Bsu)  ------------------------------------------------------   57
TatAd(Bsu)  ------------------------------------------------QDKNAG   70
TatAc(Bsu)  ------------------------------------------------EDKQM-   62
TatB(Eco)   VVKDNEAAHEGVTPAAAQTQASSPEQKPETTPEPVVKPAADAEPKTAAPSPSSSDKP  171
```

B

```
TatC(Eco)   MSVEDTQ--PLITHLIELRKRLLNCIIAVIVIFLCLVYFANDIYH-LVSAPLIK   51
TatCy(Bsu)  MTRMKVNQMSLLEHIAELRKRLLIVALAFVVFFIAGFFLAKPIIVYLQETDEAK   50
TatCd(Bsu)  MDKKETH---LIGHLEELRRRILVTLAAFFLFFLKTAFLKGDIYDWLIRDLDGK   51
            *.  ...   *..*. ***.*..     *   ....     ... *   *  .  *

TatC(Eco)   QLPQGSTMIATDVASPFFTPIKLTFMVSLILSAPVILYQVWAFIAPALYKHERR  105
TatCy(Bsu)  QL----TLNAFNLTDPLYVFMQFAFLIGIVLISPVLLYQLWAFVSPGLYEKERK  104
TatCd(Bsu)  -------LAVLGPSEILWVYMMLSGICAIAASIPVAAYQLWRFVAPALTKTERK   98
                   . . . .. ... . .. . ..  . **  **.* *..*.* . **.

TatC(Eco)   LVVPLLV---SSSLLFYIGMAFAYFVVFPLAFGFLANTAPE-GVQVSTDIASYL  155
TatCy(Bsu)  VTLSYI---PVSILLFLAGLSFSYYVLLPFFVVDFMKRISQDLNVNQVIGINEYF  155
TatCd(Bsu)  VTIMYIMYIPGLLFALFLAGISFGYFVLFPIVLSFLTHLSSG-HFETMFTADRYF  151
            ...  .         **  *..*.*...**.. .*. . . . .   . .   *.

TatC(Eco)   SFVMALFMAFGVSFEVPVAIVLLCWMGITSPEDLRKKRPYVLVGAFVVGMLLTP  209
TatCy(Bsu)  HFLLQLTIPFGLLFQMPVILMFLTRLGIVTPMFLAKIRKYAYFTLLVIAALITP  209
TatCd(Bsu)  RFMVNLSLPFGFLFEMPLVVMFLTRLGILNPYRLAKARKLSYFLLIVVSILITP  205
             *...*  ..**  *..*.  ...*  ..** .* * * *        .*..  *.**

TatC(Eco)   PDVFSQTLLAIPMYCLFEIGVFFSRF-YVGKGRNREEENDAEAESEKTEE  258
TatCy(Bsu)  PELLSHMMVTVPLLILYEISILISKAAYRKAQKSSAADRDVSSG-----Q  254
TatCd(Bsu)  PDFISDFLVMIIPLLVLFEVSVTLSAFVYKKRMRE-----ETAAA-----A  245
            *.  .*.  ..  .*.   *.*...  .*    *     ..     .....     .
```

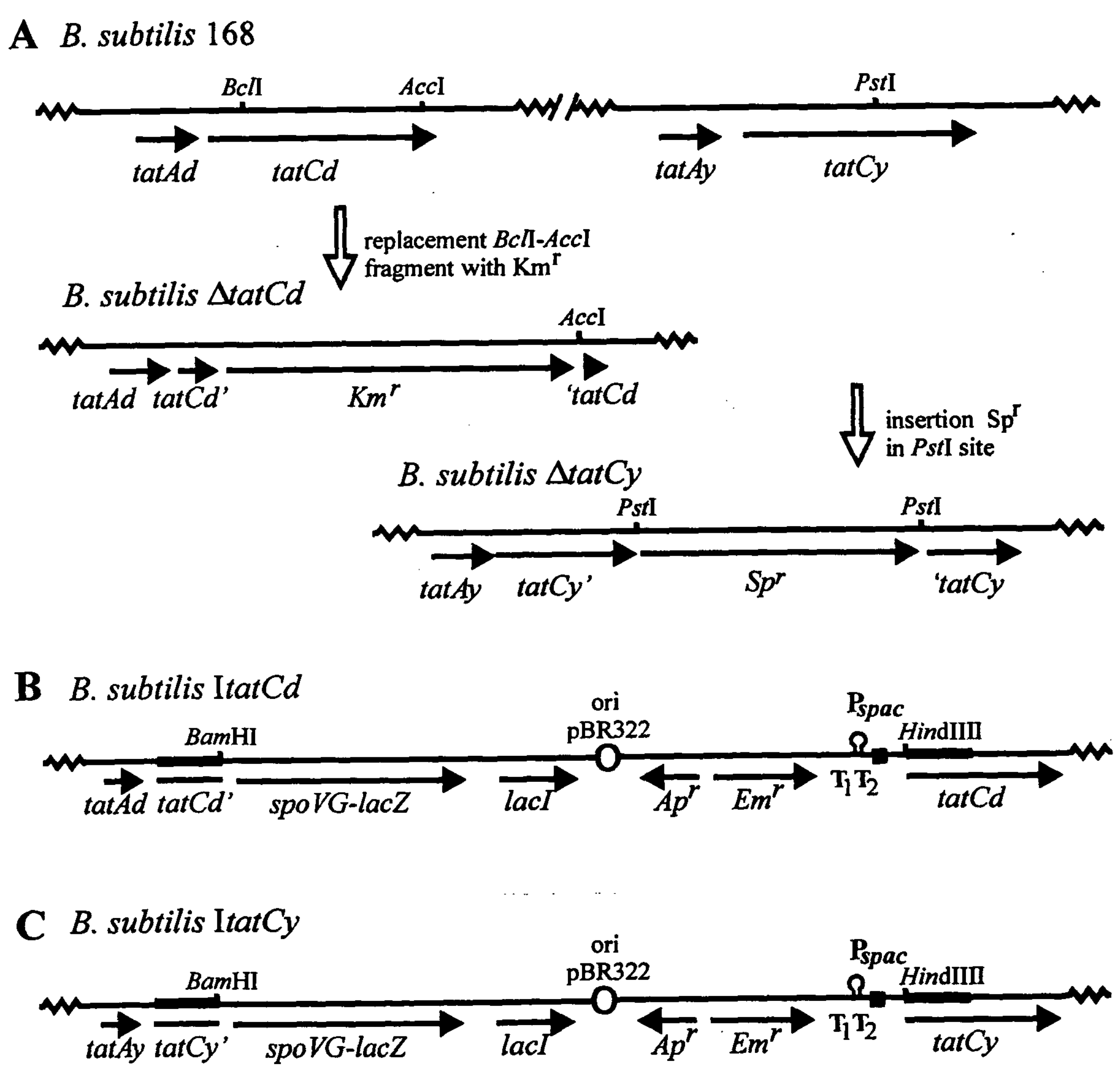
GC634-2
9/17/01
Bron et al

Fig. 2.



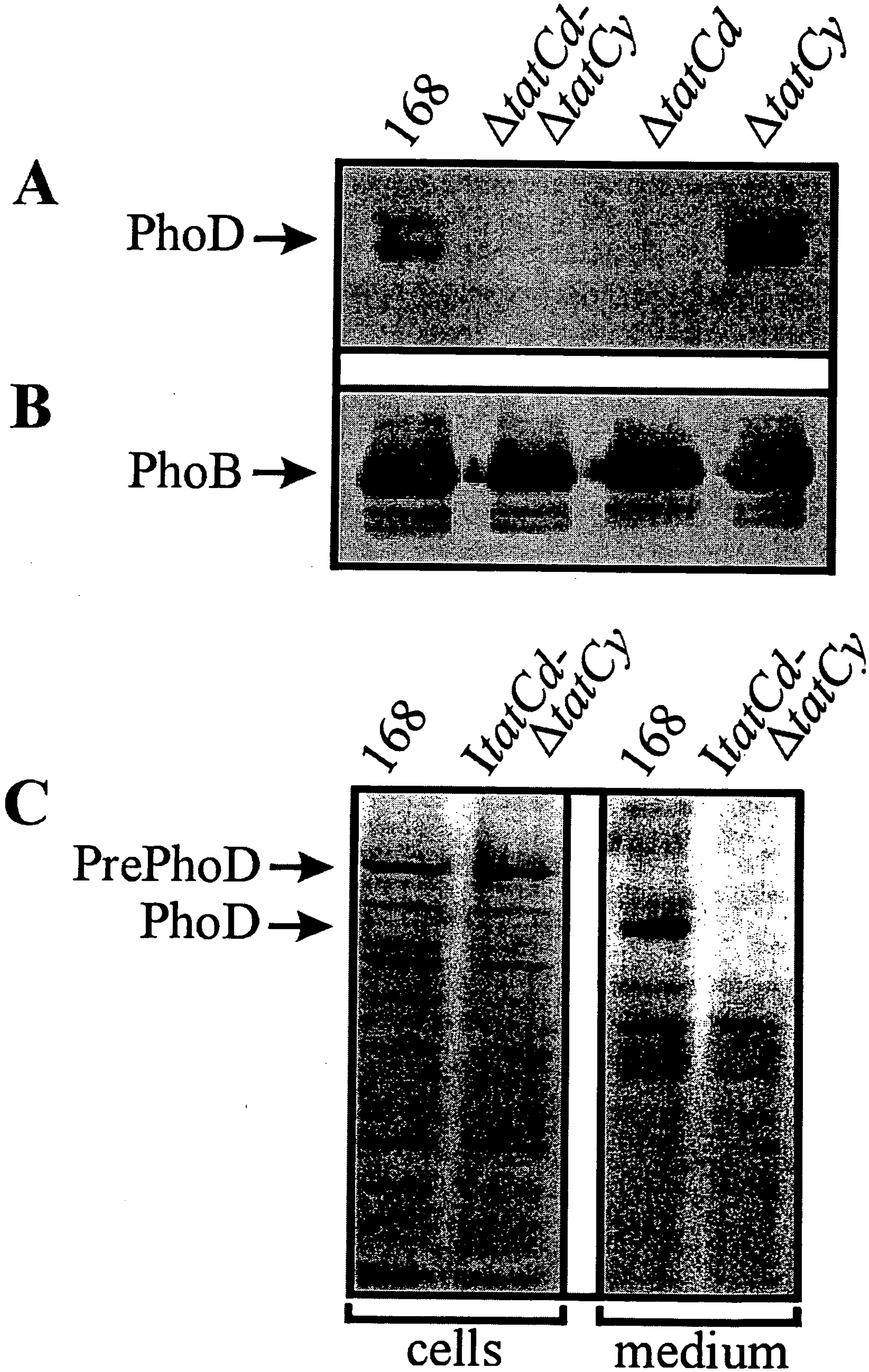
GC634-2
9/17/01
Bron et al

Fig. 3.



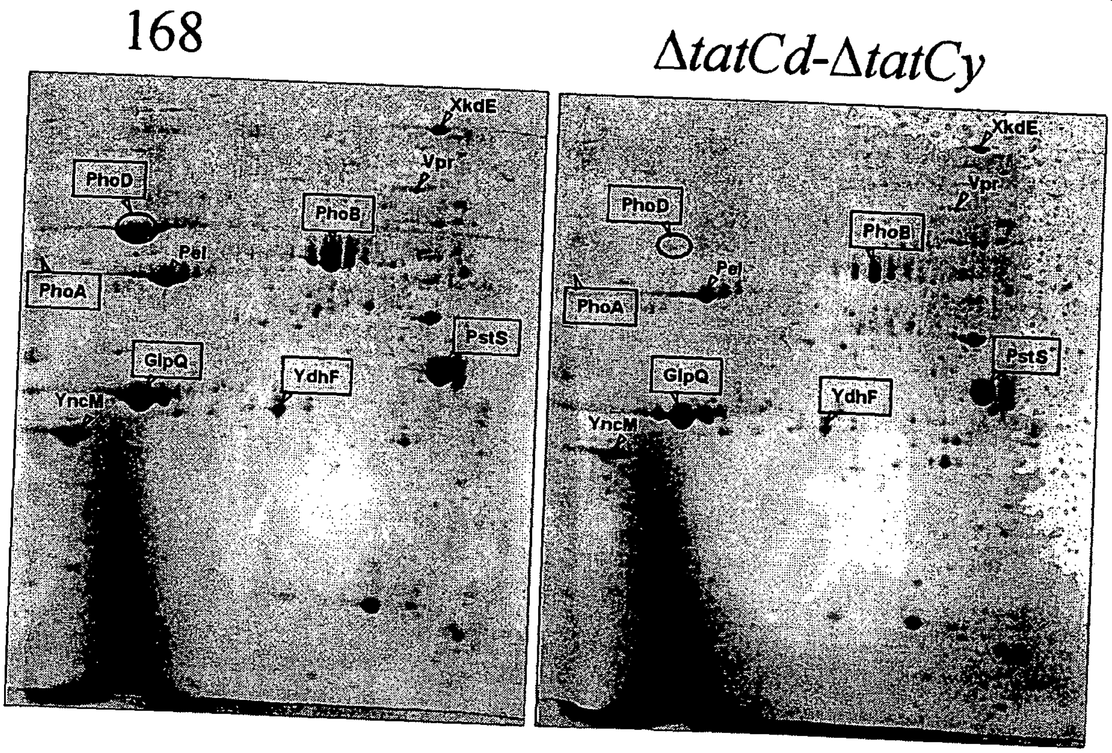
GC634-2
9/17/01
Bron et al

Fig. 4.



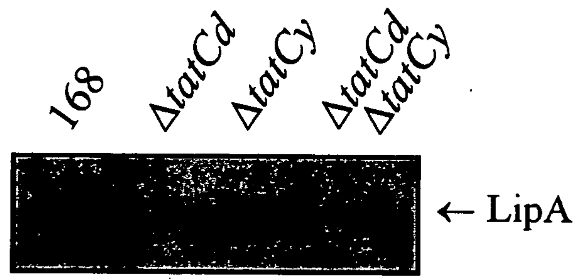
GC634-2
Bron et al
9/17/01

Fig. 5.



GC634-2
Bron et al
9/17/01

FIGURE 6



. Tat-dependent secretion of the *B. subtilis* lipase LipA. *B. subtilis* 168 (parental strain), *B. subtilis* Δ*tatCd*, *B. subtilis* Δ*tatCy*, or *B. subtilis* Δ*tatCd*-Δ*tatCy* were grown in TY-medium to end-exponential growth fase. To study the secretion of LipA, *B. subtilis* cells were separated from the growth medium by centrifugation. Proteins in the growth medium were concentrated 20-fold upon precipitation with trichloroacetic acid, and samples for polyacrylamide gel electrophoresis (SDS-PAGE) were prepared. Secreted LipA in the growth medium was visualized by SDS-PAGE and Western blotting, using LipA-specific antibodies.

GC634-2
Bron et al
9/17/01

FIGURE 7

Predicted twin-arginine (RR-)signal peptides of *B. subtilis*[1]

Protein	N	h	RR-Motif	H	h	C
AlbB	1	0.1	RRILL	27	2.0	AIA
AmyX TM	9	-0.8	RRSFE	15	1.1	-
AppB TM	8	0.5	RRTLM	19	2.3	-
LipA	7	-1.1	RRIIA	19	1.2	AKA
OppB TM	8	-0.6	RRLVY	24	2.0	-
PbpX	2	-2.2	RRRKL	14	2.9	WNA
PhoD	3	-1.3	RRKFI	17	0.9	VGA
QcrA TM	1	-1.1	RRQFL	19	1.3	-
TlpA TM	1	-0.8	RRLII	21	2.4	-
WapA W	1	-3.0	RRNFK	18	2.3	VLA
WprA	8	-1.7	RRKFS	20	1.9	AAA
YceA TM	1	-0.4	RRAFL	21	2.2	-
YesM TM	1	-1.5	RRMKI	20	2.4	QYA
YesW	1	-1.3	RRSCL	19	2.0	VKA
YfkN TM	1	-1.2	RRTHV	17	1.7	IHA
YkpC	8	-1.0	RRVAI	17	2.3	SLA
YkuE	1	-1.3	RRQFL	17	1.0	GYA
YmaC	7	0.0	RRFLL	15	2.4	YSL
YubF TM	9	-2.7	RRNTV	23	2.0	-
YuiC	8	0.2	RRLLM	20	1.9	IEA
YvhJ TM	2	-1.7	RRKIL	18	2.5	-
YwbN	1	-1.8	RRDIL	23	1.4	QTA

[1] The listed signal peptides contain, in addition to the twin-arginines, at least one other residue of the consensus sequence (R-R-X-ϕ-ϕ; printed in bold). The number of residues in the N- and H-domains of each signal peptide, and the average hydrophobicity (h) of each of these domains, as determined by the algorithms of Kyte and Doolittle (Kyte, J., and R. F. Doolittle [1982] A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. 157:105-32), are indicated. Furthermore, the RR-motifs in the N-domain, and SPase I recognition sites in the C-domain (*ie.* positions -3 to -1 relative to the predicted SPase cleavage site) are shown. Proteins lacking a (putative) SPase I cleavage site, some of which contain additional transmembrane domains, are indicated with "TM". One protein containing cell wall binding repeats is indicated with "W".

GC634-2
Bron et al
9/17/01

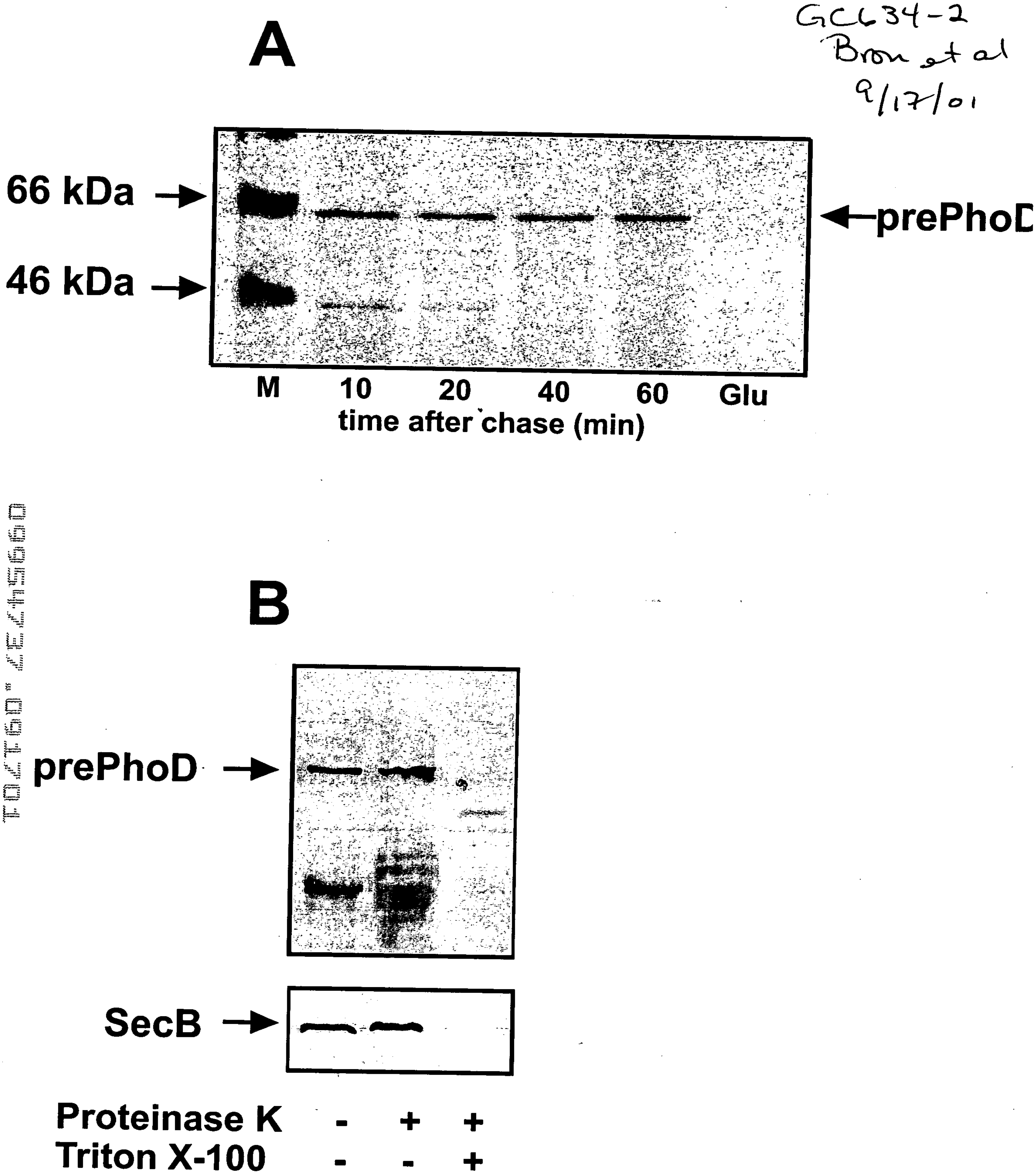


Figure 8

GC634-2
Braun et al
9/17/01

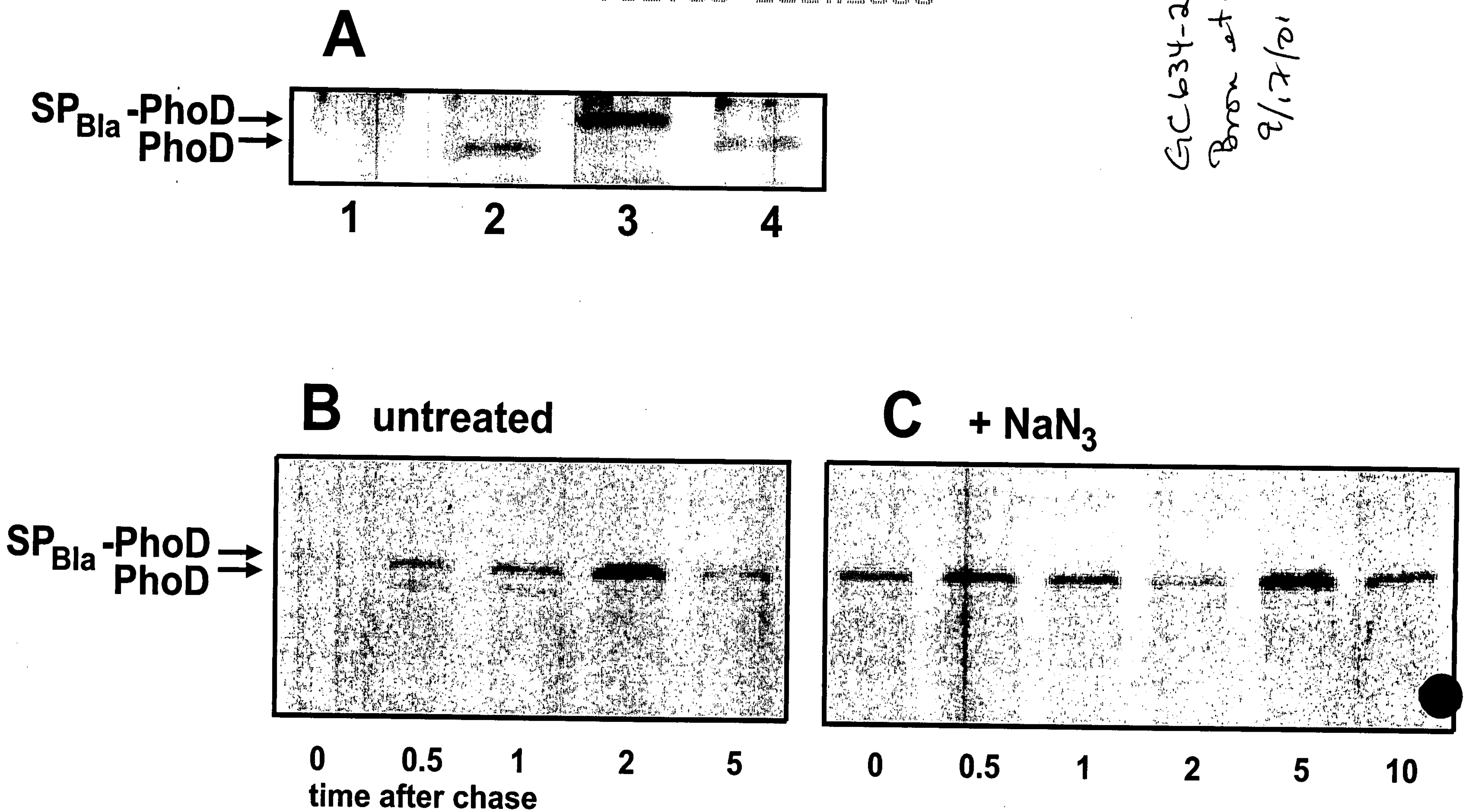


Figure 9

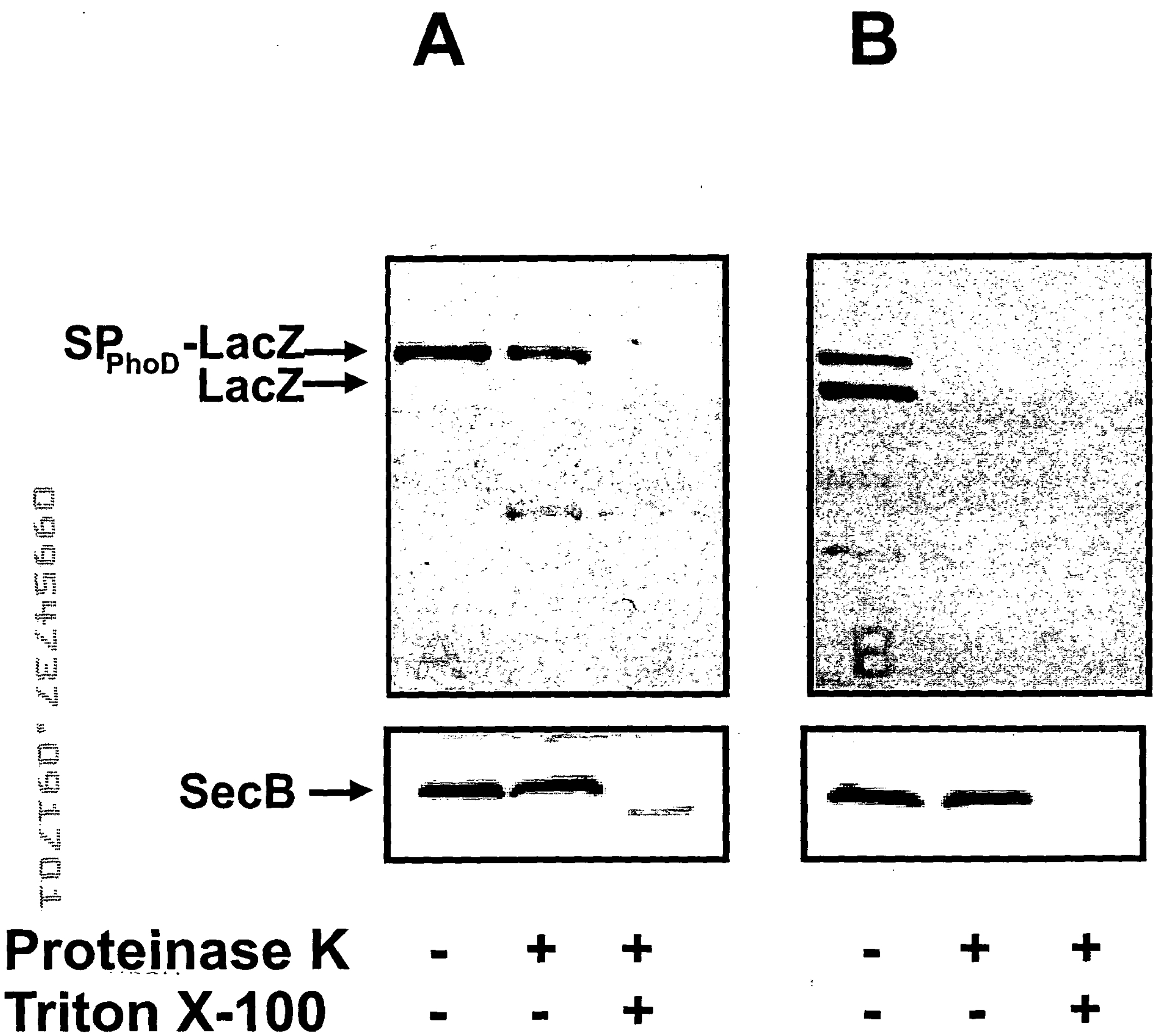


Figure 10

GC634-2
Bron et al
9/17/01

GC 634-2
Bron et al
9/12/01

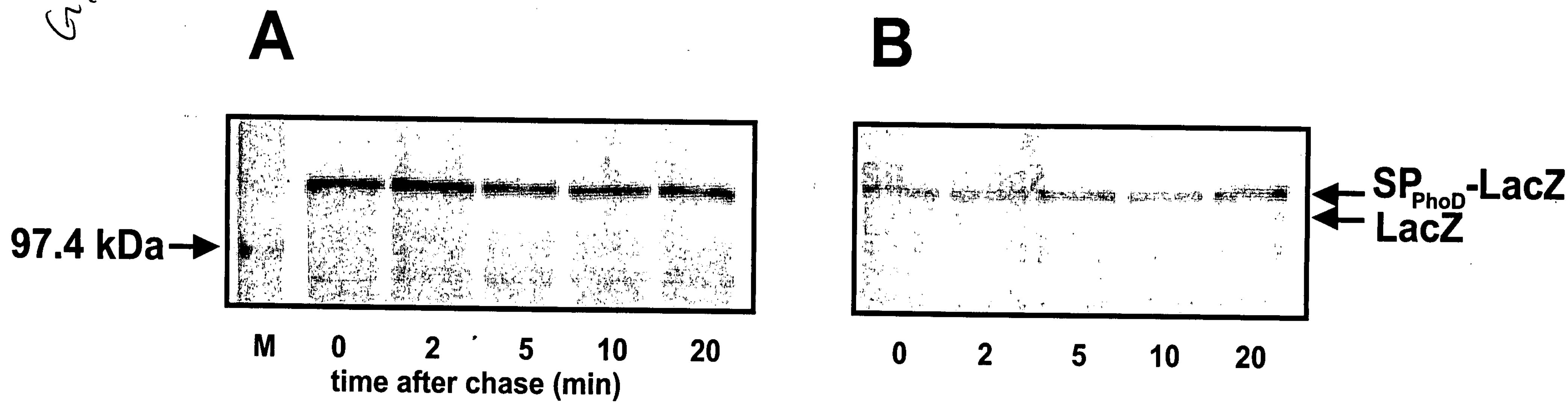


Figure 11

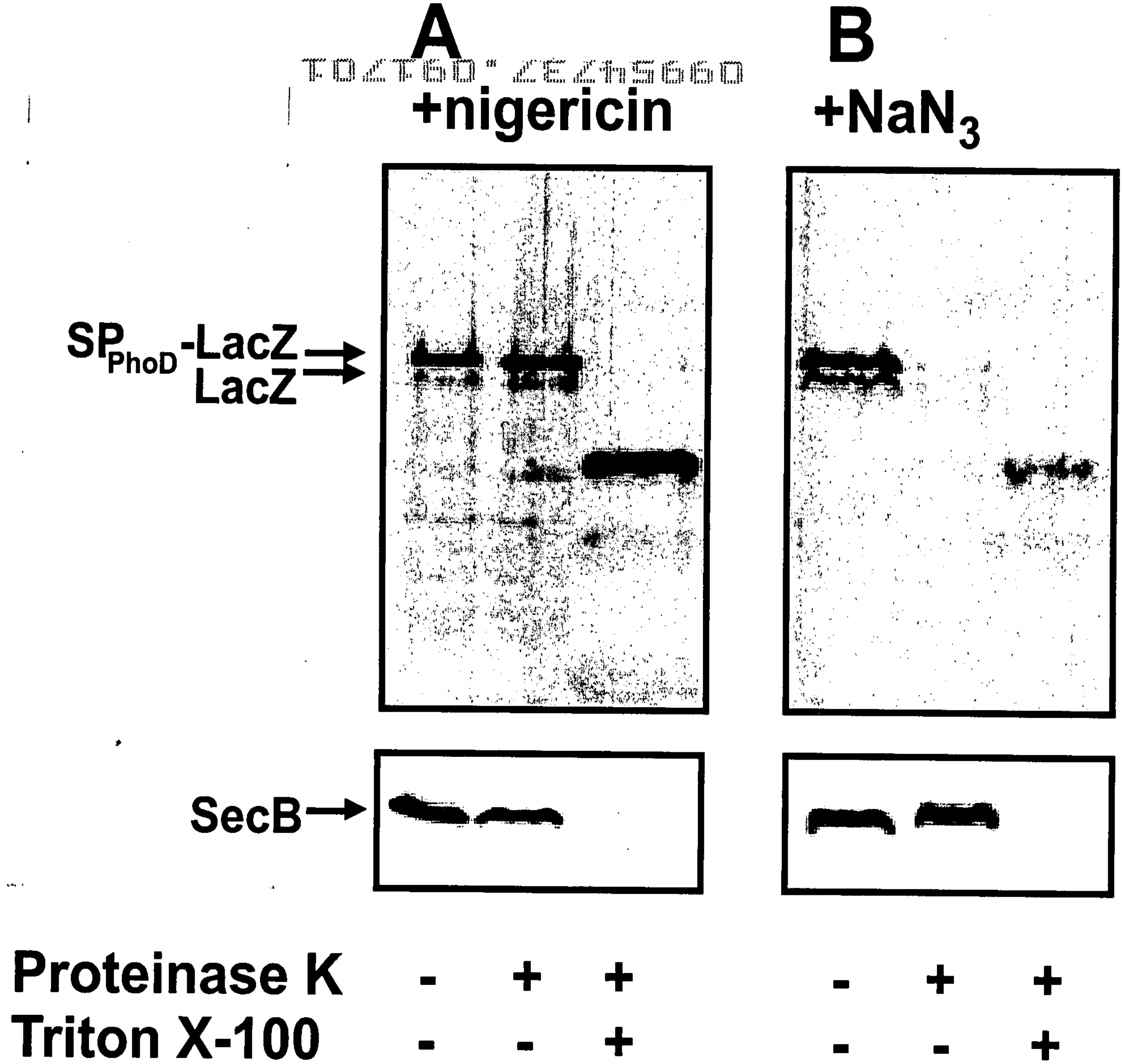


Figure 12

[redacted]on et al
GC 634-2
9/17/01

GC634-2
Bron et al
9/17/01

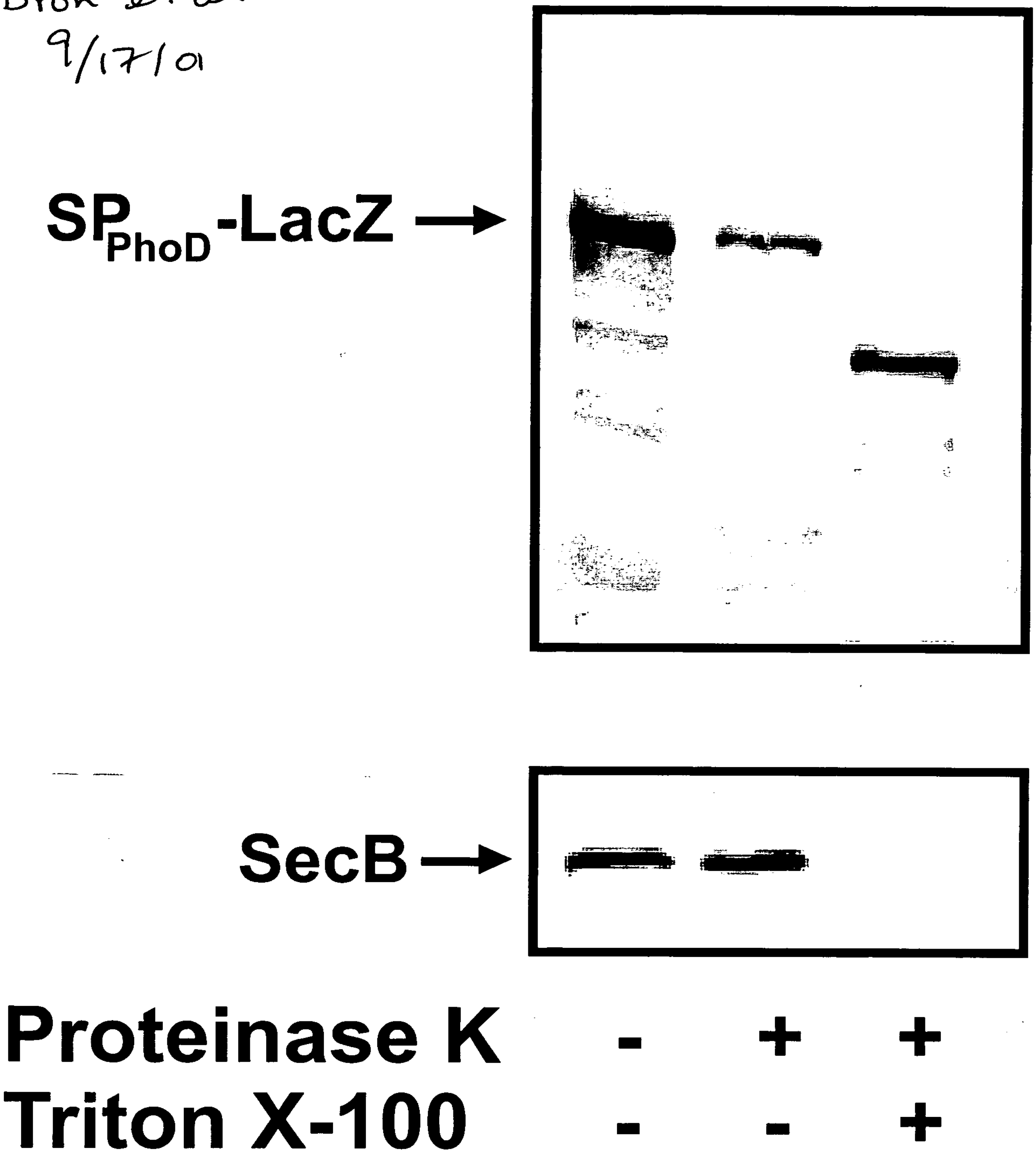


Figure 13

634-2
Bron et al
9/17/01

Figure 14
Homologs in B. alcalophilus

TatA
MGGLSVGSVVLIALVALLIFGPKKLPELGKAAGSTLREFKNATK GLADDDDDTKSTNVQKEKA

TatC
MTMMTPNQQTSKKKKRKGRKGRVPMQDMSIMDHAEELRRRIF VVLAFFIVALIGGFFLAVPVITFLQNSPQAADMPFNAFRLTDPLRV YMNFAVITALVLIIPVILYQLWAFVSPGLKENEQKATLAYIPIAFL LFLAGIAFSYFILLPFVISFMGQMADRLEINEMYGINEYFSFLFQL TIPFGLLFQLPVVVMFLTRLGVVTPTFLRKIRKYAYFALLVIAGII TPPELTSHLFVTVPMLILYEISITISAITYRKYHGTTDHNGQESAK